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Ascotricha microspora sp. nov. from Cayman Islands

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Abstract—A new hyphomycete species, *Ascotricha microspora* collected from Cayman Islands in the Caribbean, is described and illustrated. The asexual fungus differs from *Dicyma vesiculifera* by having smaller, colorless conidia. *Ascotricha rugispora* (≡ *Surculiseries rugispora*) and *A. funiculosa* (≡ *Dicyma funiculosa*) are proposed as new combinations.

Key words—ITS, microfungi, phylogeny, saprobe, *Xylariaceae*

Introduction

Ascotricha Berk. was proposed as a monotypic ascomycete genus (Berkeley 1838) typified by *A. chartarum* Berk. Boulanger (1897) typified *Dicyma* Boulanger by *Dicyma ampullifera* Boulanger, which he described as the conidial state of *Chaetomium zopfii* Boulanger [≡ *Ascotricha zopfii* (Boulanger) Peyronel], all names that were later synonymized with *Ascotricha chartarum* (Hawksworth 1971). Von Arx (1982) concluded that *Dicyma* is synonymous with *Hansfordia* S. Hughes, *Basifimbria* Subram. & Lodha, *Gonytrichella*, and *Puciola* De Bert. Nonetheless, some mycologists still regard *Hansfordia* and *Basifimbria* as distinct genera (Seifert & al. 2011). As the type species of *Dicyma* 1897 (*D. ampullifera*) and *Ascotricha* 1838 (*A. chartarum*) are

Table 1. ITS sequences of *Ascotricha microspora* and allied taxa used for phylogenetic analysis.

Taxon	Accession no.*	Substrate	Locality	ITS	Reference
Ascotricha bosei	CBS448.93 [T]	Date palm plantation soil	Iraq	KF893283	Cheng & al. 2015
Ascotricha chartarum	CBS234.97	Tropical rain forest soil	Papua New Guinea	KF893284	Cheng & al. 2015
	B4C	Oil on canvas painting	Nigeria	KX246915	Okpalanozie & al. 2016
	B4F	Oil on canvas painting	Nigeria	KX246917	Okpalanozie & al. 2016
Ascotricha erinacea	S98	*Dendrobium officinale*	China	KT224876	—
	CBS535.73	Elephant dung	East Africa	KF893285	Cheng & al. 2015
	CBS407.87	Paper and cardboard	Netherlands	KF893287	Cheng & al. 2015
	OUCMBIII$_{10}$1069	Sediment	China	KF893281	Cheng & al. 2015
	NBRC31999	Leaf of broad-leaved tree	Israel	03199901	—
	NBRC9841	Urethane foam	—	00984101	—
	NBRC33232	House dust	Japan	03323201	—
Ascotricha guamensis	NBRC9991 (IMI 82619)	Soil	—	999103	—
Ascotricha longipila	CGMCC 3.15229 (OUCMB$_{01}$18) [T]	Marine algae	China	KC503896	Cheng & al. 2015
Ascotricha lusitanica	S19	*Dendrobium officinale*	China	KT224797	—
	CBS462.70 [IT]	*Cicer arietinum* seed	Portugal	KF893289	Cheng & al. 2015
Ascotricha microspora	NHES L1706 [T]	Unidentified decaying leaf	Cayman Islands	MF805818	This study
Ascotricha parvispora	CGMCC 3.14995 (OUCMBI$_{11}$0001) [T]	Living *Enteromorpha linza*	China	JX014298	Cheng & al. 2015
Ascotricha sinuosae	CGMCC3.149965 (OUCMBI$_{12}$1190) [T]	Living *Colpomenia sinuosa*	China	JX014299	Cheng & al. 2015
	A1S5-10	Beach soil	Malaysia	KJ780756	—
	F8	Plant endophyte	Sudan	KR673905	—
Daldinia bambusicola	E40F	Agarwood tree	India	KY425743	—
Daldinia childiae	2-00219-2	*Platycladus orientalis* leaf	China	KT192354	—
Daldinia eschscholtzii	JMRC SF 11930	*Paphiopedilum exul* root	Thailand	KU304335	Barnes & al. 2016

Daldinia petriniae	H Knudsen s.n. (C)	*Corylus avellana*	Denmark	AF176970	Johannesson & al. 2000
Dicyma funiculosa	CBS124.80 [T]	Soil	Spain	KF893286	Cheng & al. 2015
	CBS323.86	Forest soil	Spain	AY908992	U'Ren & al. 2016
Dicyma pulvinata	414-3	—	—	LC177968	Unpublished
	KACC44502	—	—	LC177971	Unpublished
	CBS194.56	*Prunus persica* twig	Italy	AY908993	U'Ren & al. 2016
	LCC11	Giant panda vaginal secretions	China	KF986550	—
	5023	*Eucalyptus globulus* leaves	Spain	FR667996	Sánchez Márquez & al. 2011
	CBS254.59	*Lycopersicon esculentum* leaf	Netherlands	AY908995	Peláez & al. 2008
Hansfordia nebularis	CBS115626	*Quercus robur*	Italy	KF893290	Cheng & al. 2015
Hansfordia pulvinata	HMQAU130012	Tomato leaf?	China	KP772241	—
	KACC44498	Hyperparasitizing *Passalora fulva* on organic tomato plants	Korea	HM060587	—
	NBRC30211	*Lithocarpus edulis*	Japan	03021101	—
	NBRC6791	—	—	00679101	—
	NBRC9007	—	—	00900701	—
Lopadostoma turgidum	CBS 133207 [T]	*Fagus sylvatica*	Austria	NR132036	Jaklitsch & al. 2014
Podosordaria muli	WSP167 [T]	Mule dung	Mexico	GU324761	Hsieh & al. 2010
Surculiseries rugispora	NBRC33167 [T]	*Bruguiera gymnorhiza* leaf	Japan	LC146763	Okane & al. 2001
	NBRC33168	*Bruguiera gymnorhiza* leaf	Japan	03316801	Okane & al. 2001
Xylaria hypoxylon	ATCC42768 [T]	Wood	USA	AY327477	Platas & al. 2004

* [T] = ex-holotype; [IT] = ex-isotype.

ATCC = American Type Culture Collection, Manassas, United States; **BICC** = Biocon culture collection, India; **BCC** = BIOTEC Culture Collection, Thailand; **CBS** = Westerdijk Fungal Biodiversity Institute (formerly Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; **HMQAU** = Fungal Herbarium of Qingdao Agricultural University, Qingdao, China; IMI = IMI Fungarium, the Royal Botanic Gardens, Kew, UK; **KACC** = Korean Agricultural Culture Collection, South Korea; **MFLUCC** = Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; **NBRC** = the NITE Biological Resource Center, Japan; **NFCCI** = the National Fungal Culture Collection of India; **NRRL** = The Agricultural Research Service Culture Collection, USDA, USA; **OUCMB** = Herbarium of Ocean University of China, Marine Biology, Shandong, China; **UAMH** = UAMH Centre for Global Microfungal Biodiversity, University of Toronto, Canada; **WSP** = Washington State University, Pullman, Washington, U.S.A.

synonyms (Hawksworth 1971), the generic names are likewise synonymous, with *Ascotricha* having priority as the earlier name.

Here we describe a new asexual species in *Ascotricha*, collected from Cayman Islands.

Materials & methods

The fungus was collected from fallen leaves and isolated on malt extract agar and incubated at 25°C. Conidiophores, conidiogenous cells and conidia were mounted in 85% lactic acid. A staining agent, 0.1% lacto-fuchsin, was used to observe conidiogenous cells and septation of colorless conidia. All microscopic observations were made under Nomarski differential interference contrast optics of Olympus BX40 microscope. Photomicrographs were taken with an Olympus Microfire digital camera. Measurements of the fungal structures were statistically analyzed with Microsoft Office Excel 2013 with 95% confidence interval of means. The results are presented as ranges calculated based on mean ± standard deviation with n = number of fungal structures measured. The holotype specimen is conserved in the Herbarium, Connecticut Agricultural Experiment Station, New Haven, U.S.A. (NHES).

DNA extraction and ITS sequencing procedures followed Li & al. (2008) as carried out by Gen Script (Nanjing) Co., Ltd. (Nanjing, China). The ITS sequence underwent BLAST analysis via the NBCI web site (www.ncbi.nlm.nih.gov/blast/) for sequence similarity searches and comparisons. Forty-six sequences representing *Ascotricha*, *Dicyma*, *Hansfordia*, and several genera of *Xylariaceae* and allied families were chosen for phylogenetic analysis (Table 1). Sequences are aligned with Guidance2 (mafft) (http://guidance.tau.ac.il/ver2/). *Lopatostoma tugidum* CBS 133207 was included as outgroup. The ITS sequence has been deposited in GenBank. Phylogenetic analysis was conducted using MEGA7 (Kumar & al. 2016). Bootstrap test was carried out with 1000 replicates.

Results

Our phylogenetic analysis distinguished *A. microspora* from other *Ascotricha*/*Dicyma* species and supported it as sister to *Surculiseries rugispora*. As can be seen in Fig. 1, *Ascotricha microspora*, *A. bosei*, *A. chartarum*, *A. erinacea* [= *Dicyma olivacea*], *A. guamensis*, *A. longipila*, *A. lusitanica*, *A. parvispora*, *A. sinuosae* [≡ *Hansfordia sinuosae*], *Dicyma funiculosa,* and *Surculiseries rugispora* group within the same clade (the /Ascotricha clade) with 97% support; the inclusion of the ex-type sequence of the monotypic genus *Surculiseries* within /Ascotricha suggests that it is congeneric with *Ascotricha*. Fig. 1 shows all *Dicyma funiculosa* sequences as monophyletic with 99% support and all sequences of *Ascotricha erinacea* [= *Dicyma olivacea*] as monophyletic with 98% support. However, the *Ascotricha chartarum* sequences are not monophyletic, with two sequences (B4C and B4F) grouping

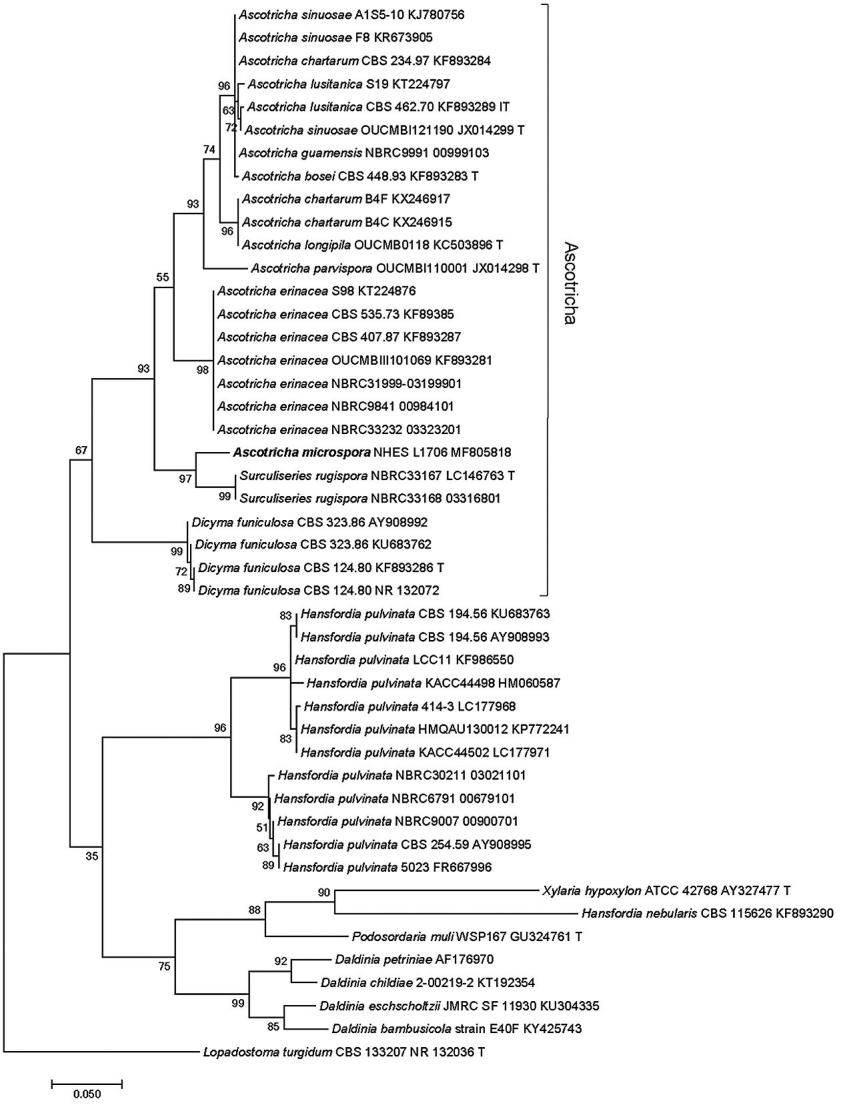

Ascotricha sinuosae A1S5-10 KJ780756
Ascotricha sinuosae F8 KR673905
Ascotricha chartarum CBS 234.97 KF893284
Ascotricha lusitanica S19 KT224797
Ascotricha lusitanica CBS 462.70 KF893289 IT
Ascotricha sinuosae OUCMBI121190 JX014299 T
Ascotricha guamensis NBRC9991 00999103
Ascotricha bosei CBS 448.93 KF893283 T
Ascotricha chartarum B4F KX246917
Ascotricha chartarum B4C KX246915
Ascotricha longipila OUCMB0118 KC503896 T
Ascotricha parvispora OUCMBI110001 JX014298 T
Ascotricha erinacea S98 KT224876
Ascotricha erinacea CBS 535.73 KF89385
Ascotricha erinacea CBS 407.87 KF893287
Ascotricha erinacea OUCMBIII101069 KF893281
Ascotricha erinacea NBRC31999-03199901
Ascotricha erinacea NBRC9841 00984101
Ascotricha erinacea NBRC33232 03323201
Ascotricha microspora NHES L1706 MF805818
Surculiseries rugispora NBRC33167 LC146763 T
Surculiseries rugispora NBRC33168 0331680l
Dicyma funiculosa CBS 323.86 AY908992
Dicyma funiculosa CBS 323.86 KU683762
Dicyma funiculosa CBS 124.80 KF893286 T
Dicyma funiculosa CBS 124.80 NR 132072
Ascotricha
Hansfordia pulvinata CBS 194.56 KU683763
Hansfordia pulvinata CBS 194.56 AY908993
Hansfordia pulvinata LCC11 KF986550
Hansfordia pulvinata KACC44498 HM060587
Hansfordia pulvinata 414-3 LC177968
Hansfordia pulvinata HMQAU130012 KP772241
Hansfordia pulvinata KACC44502 LC177971
Hansfordia pulvinata NBRC30211 03021101
Hansfordia pulvinata NBRC6791 00679101
Hansfordia pulvinata NBRC9007 00900701
Hansfordia pulvinata CBS 254.59 AY908995
Hansfordia pulvinata 5023 FR667996
Xylaria hypoxylon ATCC 42768 AY327477 T
Hansfordia nebularis CBS 115626 KF893290
Podosordaria muli WSP167 GU324761 T
Daldinia petriniae AF176970
Daldinia childiae 2-00219-2 KT192354
Daldinia eschscholtzii JMRC SF 11930 KU304335
Daldinia bambusicola strain E40F KY425743
Lopadostoma turgidum CBS 133207 NR 132036 T
0.050

Fig. 1. Maximum Likelihood analysis of *Ascotricha microspora* and allied taxa, based on 46 sequences. *Lopatostoma tugidum* CBS 133207 is included as outgroup. The bootstrap test was conducted with 1000 replicates; bootstrap values >50% are indicated at the nodes. The scale bar indicates the number of expected changes per site. Annotations T and IT indicate the ex-type sequences included in the analysis.

with *A. longipila* while the third (CBS234.97) groups with *Ascotricha bosei*, *A. guamensis*, *A. lusitanica*, and *A. sinuosae* [≡ *H. sinuosae*].

All 12 isolates of *Hansfordia pulvinata* [≡ *Dicyma pulvinata*] were monophyletic with 96% support and sister to a clade (containing sequences of *Daldinia* and other genera) within which *H. nebularis* grouped with *Xylaria hypoxylon* (Fig. 1). The clear separation between these clades and the /Ascotricha clade indicates that *H. pulvinata* and *H. nebularis* do not belong in *Ascotricha*.

Taxonomy

Ascotricha Berk., Annals of Natural History 1: 257 (1838)

= *Dicyma* Boulanger, Revue Générale de Botanique 9: 18 (1897)

= *Gonytrichella* Emoto & Tubaki, Transactions of the Mycological Society of Japan 11: 95 (1970)

= *Puciola* De Bert., Mycotaxon 3(3): 553 (1976)

= *Surculiseries* Okane, Nakagiri & Tad. Ito, Mycoscience 42(1): 116 (2001)

Ascotricha microspora D.W. Li & G.H. Zhao, **sp. nov.** Fig. 2

MycoBank MB 822539

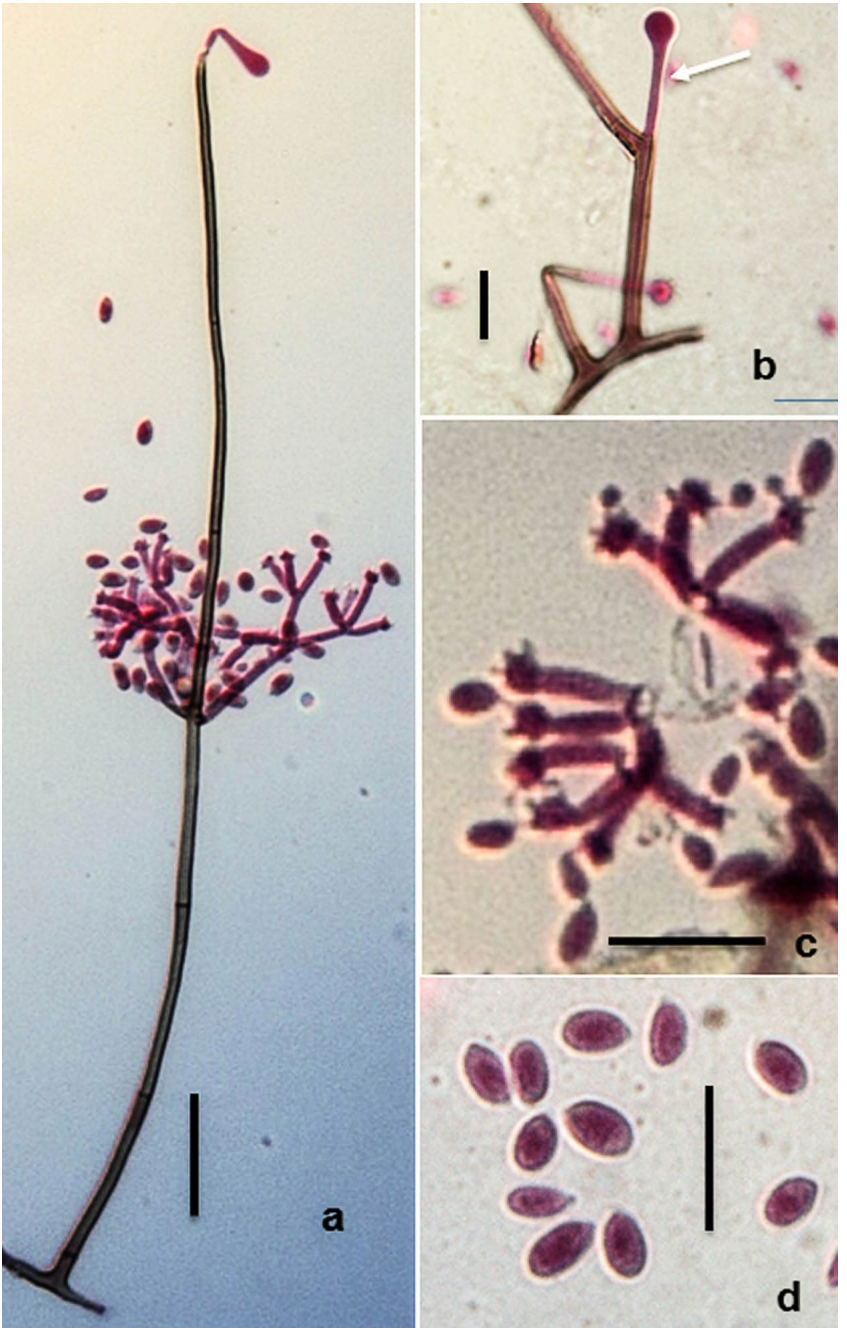
Differs from *Dicyma vesiculifera* by its smaller, colorless conidia.

Type: Cayman Islands, Grand Cayman, fallen leaves, 2 January 2012, De-Wei Li (**Holotype**, NHES L1706 (GenBank MF805818).

Etymology: epithet means small conidia.

Colonies hypophyllous, effused, lanose, gray. Mycelium partly superficial, partly immersed in the substrate, composed of colorless to pale brown hyphae, 1–2 µm diam. Conidiophores differentiated, simple, solitary, ≤220 µm long, 2.5–3.2 µm diam, smooth, erect or geniculate, alternately branched, brown to pale brown, gradually tapering toward the apex, septate, usually terminating in a capitate or spathulate cell and often developing a similar cell at each geniculation. The capitate or spathulate cells (drumstick cells) are sterile, colorless, smooth, thin-walled, (10.8–)12–15.4(–17.1) × (1.3–)1.4–2(–2.4) µm (mean ± SD = 13.7 ± 1.6 × 3.8 ± 0.6 µm, n = 12), with an apex enlarged into globose or subglobose shaped vesicle, (2.9–)3.2–4.4(–4.9) µm diam. (mean ± SD = 3.8 ± 0.6 µm, n = 12). Lateral branches arising singly, in pairs or in whorls, penicillate, colorless or pale brown below and colorless above, thin walled, smooth, up to 50 µm long, sometimes developing a thin-walled

Fig. 2. *Ascotricha microspora* (holotype, NHES L0706). a. Conidiophore, conidiogenous cells, and conidia; b. Partial conidiophore and a drumstick cell at geniculation (arrowed); c. Conidiogenous cells and conidia; d. conidia. Scale bars: a = 20 µm; b–d = 10 µm.


a
b
c
d

ovoid or pyriform, colorless vesicle laterally just below secondary branches, (4.6–)5.3–7.8(–8.7) × (2.5–)3–5(–6) µm, (mean ± SD = 6.6 ± 1.2 × 4 ± 1 µm, n = 10). Conidiogenous cells polyblastic, cylindrical, colorless, smooth, thin-walled, (5–)6–8(–10) × (1.3–)1.4–1.7(–1.9) µm, (mean ± SD = 7 ± 1 × 1.57 ± 0.16 µm, n = 21), denticulate, enlarged at the apex bearing a cluster of 3–6 denticles, 0.5–1.4 × 0.5–0.9 µm; conidial secession schizolytic. Conidia solitary, ovoid or ellipsoid, rounded at the apical end, with a papilate basal end, unicellular, smooth, hyaline, (3.7–)4.2–4.8(–5) × (2.1–)2.4–2.8(–3) µm, (mean ± SD = 4.5 ± 0.3 × 2.6 ± 0.2 µm, n =30).

Discussion

Ascotricha microspora is morphologically rather similar to *Dicyma vesiculifera* Piroz. but differs in conidial color and size, with *D. vesiculifera* distinguished by pale yellow-brown and longer (5–7 µm) conidia; Pyrozynski 1972). Both species produce conidiogenous cells that are enlarged significantly at the apical end and bear denticles. Conidiogenesis in both species is somewhat similar to that found in *Hansfordia*, and their conidiophores develop thin-walled, colorless capitate/spathulate cells that resemble those in *Dicyma*. Thus, *Ascotricha microspora* and *D. vesiculifera* have a morphology intermediate between those in typical *Dicyma* and *Hansfordia* species.

Surculiseries was erected and typified with *S. rugispora* based on its polyblastic, backward-curving conidiogenous cells with conspicuous scars on the flattened denticle tops and ITS and LSU sequence analysis (Okane & al. 2001). *Surculiseries* remains monotypic but shares many morphological characters with *Ascotricha* [= *Dicyma*]. However, *S. rugispora* differs from *A. microspora* in conidial morphology and conidiogenous cells. The phylogenetic analysis of Okane & al. (2001) supports *Surculiseries* as paraphyletic to *Ascotricha* and its asexual state, *Dicyma*. However, our ITS-based sequence analysis supports *Ascotricha* and *Surculiseries* as congeneric (Fig. 1). LSU and SSU phylogenies generated from using available GenBank and NBRC sequences confirm the results of the ITS phylogenetic analysis (unpublished data). We therefore propose a new combination of *S. rugispora* in *Ascotricha:*

Ascotricha rugispora (Okane, Nakagiri & Tad. Ito)
D.W. Li & G.H. Zhao, **comb. nov.**
MycoBank MB 822541
≡ *Surculiseries rugispora* Okane, Nakagiri & Tad. Ito, Mycoscience 42(1): 121 (2001)

Our phylogenetic analysis (Fig. 1) also supports *Dicyma funiculosa* in the /Ascotricha clade. Consequently, we propose a new combination of *Dicyma funiculosa* in *Ascotricha:*

Ascotricha funiculosa (Guarro & Calvo) D.W. Li & G.H. Zhao, **comb. nov.**

MycoBank MB 824983

≡ *Dicyma funiculosa* Guarro & Calvo, Nova Hedwigia 37(4): 641 (1983)

The type species of *Hansfordia* is *H. ovalispora* S. Hughes (Hughes 1951), with no available culture or sequences. The phylogenetic relationship between *Dicyma* and *Hansfordia* remains unresolved. That is also the case with *Basifimbria aurea* Subram. & Lodha, the type species of *Basifimbria* (Subramanian & Lodha 1968). Since there are no sequence data of *Basifimbria* available, we cannot establish whether *Basifimbria* is congeneric with *Dicyma* or *Hansfordia*. Further studies are necessary to determine the phylogenetic relationships among the three genera.

Ascotricha sinuosae, *A. lusitanica*, *A. guamensis*, and *A. bosei* (together with one sequence labelled as "*A. chartarum*") share the same clade, with 96% support. Potential synonymies cannot be resolved because of the lack of support within this clade, and the delineation of the three species needs to be further studied with multiple genes.

The current phylogenetic analyses suggest that *Hansfordia pulvinata* and *H. nebularis* may not belong to *Ascotricha* (Fig. 1), and their placement is yet to be determined.

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